REPORT TITLE

Toxicology Response by the Endosulfan Task Force to the Health Effects Division Risk Assessment for the Endosulfan Reregistration Eligibility Decision Document Dated January 31, 2001:

Selection of a Dermal No Observable Adverse Effect Level (NOAEL)

DATA REQUIREMENT

Not Applicable

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REPORT IDENTIFICATION

B003505

REPORT DATE

October 19, 2001

SPONSOR/SUBMITTER

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SUBMISSION VOLUME

Volume 1 of 3

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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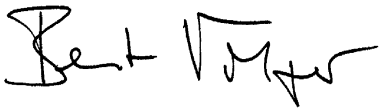
STATEMENT OF GOOD LABORATORY PRACTICE

No Good Laboratory Practice Statement is required for the information presented in this volume according to 40CFR Part 160.



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HEALTH EFFECTS DIVISION (HED) RISK ASSESSMENT
FOR THE ENDOSULFAN REREGISTRATION ELIGIBILITY DECISION
DOCUMENT, DATED JANUARY 31, 2001

TOXICOLOGY CHAPTER

RE: Endosulfan: HED Risk Assessment for the Endosulfan RED Document (DP Barcode: D250471; Memo by Diana Locke, Ph.D., dated January 31, 2001) - Exposure Assessment, Section 3.0 "Hazard Characterization" and Related Documents;

Endosulfan 079401: Toxicology Chapter for the Reregistration Eligibility Document (HED memo by Nicole C. Paquette, Ph.D. dated November 22, 1999.

The Endosulfan Task Force (ETF), comprised of Aventis CropScience, FMC, and Makhteshim-Agan North America, respectfully submit the following volume in response to the above referenced chapter. This volume specifically addresses the selection of an appropriate dermal No Observable Adverse Effect Level (NOAEL) for the assessment of risk from occupational exposure and the potential of endosulfan to bioaccumulate in mammalian systems.

I. INTRODUCTION

In preparation for the final Reregistration Eligibility Decision (RED) on the active ingredient endosulfan, the EPA Health Effects Division (HED) provided the Endosulfan Task Force (ETF) with a draft of their human health risk assessment for all registered uses of this chemical. Supporting documents for this risk assessment included the Hazard Identification Assessment Review Committee (HIARC) Toxicology Chapter, the HIARC report on toxicological endpoints for risk assessment, and the FQPA Safety Factor Committee report. On May 10, 2000 and January 5, 2001, the ETF submitted an initial 30-day and subsequent response, respectively, identifying errors in the draft risk assessment and providing brief summaries on issues of concern regarding the hazard characterization of endosulfan.

The purpose of this submission is to further elucidate the areas of concern discussed briefly in the previous ETF responses. One of the most significant issues to be discussed by the ETF is the selection of the dermal No Observable Adverse Effect Level (NOAEL) for use in occupational exposure scenarios and risk assessments. A second issue that will be discussed in this response is the potential of endosulfan to bioaccumulate in mammalian systems, and whether an additional uncertainty factor is required to assess intermediate/long-term occupational exposures.

The ETF concurs with the HIARC's selection of the 21-day dermal toxicity study for providing the most appropriate data for dermal short-term and intermediate/long-term exposure assessments. However, the ETF does not agree with the HIARC's determination of the NOAEL resulting from its review of the available data, nor the conclusion that endosulfan bioaccumulates, representing potentially increasing risk with longer-term exposures. The remainder of this document will provide a detailed review of the available data, a weight-of-evidence evaluation of the dermal toxicity and pharmacokinetics of endosulfan, and a full rationale for selection of an appropriate toxicity endpoint for dermal exposure assessments.

II. NOAEL selection for the 21-day dermal study in rats

A. EPA Conclusion

The EPA HED chapter provided the following conclusion on selection of a dermal toxicity endpoint for dermal exposure assessments. *"The dose and endpoint selected for risk assessment was dermal NOAEL= 3 mg/kg/day, based on mortality with clinical signs in males, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg/day (LOAEL). This 21-day dermal study is appropriate for dermal exposure scenarios up to 30 days. The toxicity endpoint is supported by another 21-day dermal toxicity study (MRID 41048505) in which clinical signs (tremors, straub-tail, spasms) and mortality occurred in female rats treated dermally with 12 mg/kg of a formulation (33.3% ai) of endosulfan. A Margin of Exposure (MOE) of 100 (10x for inter-species extrapolation and 10x for intra-species variability) is adequate for occupational exposure."*[1] *"The 21-day dermal toxicity study in rats (ACC.# 257684/257685) was also selected for intermediate/long-term dermal exposure."*[1]

B. ETF Response

The ETF agrees with the HIARC selection of the 21-day dermal study, but does not agree with the assessment of the NOAEL. The ETF addressed this issue in detail in a response submitted January 5, 2001 (MRID 45300201). Based on the weight-of-evidence determination provided in the above referenced response, the ETF believes that the most appropriate dermal NOAEL is 12 mg/kg bw/day based on increased mortality in female rats. This determination is well supported by all of the dermal toxicity studies, as well as available information from subchronic oral studies.

[1] Locke D., Endosulfan: HED Risk Assessment for the Endosulfan Reregistration Eligibility Decision (RED) Document. Chemical No. 079401. Case No. 0014. Barcaode D250471. January 31, 2001

The ETF evaluated five subchronic dermal toxicity studies on endosulfan in making a weight-of-evidence determination. The two main subchronic 21-day dermal toxicity studies in rats, using technical material, were conducted according to EPA guidelines (MRID 00146841 & 00147744), the third is a non-guideline study in the public literature (Dikshith et al. 1988, Appendix 3). In addition, the ETF also evaluated two 21-day dermal toxicity studies using formulated endosulfan, 49.5% technical in a wettable powder (MRID 41048506) and an emulsifiable concentrate formulation at 33.3% technical (MRID 41048505). These formulated product studies can not be directly correlated to the results from technical material studies, however, they can provide supportive evidence for selection of a dermal toxicity endpoint.

Study Number	MRID	Formulation	Doses (mg/kg bw/day)	
			Male	Female
A30754	00147744	Technical	0, 12, 48, 96, 192	0, 3, 6, 12, 48
A30753	00146841	Technical	0, 1, 3, 9, 27, 81	0, 1, 3, 9, 27
A41365	Dikshith, T.S.S.	Technical	18.8, 37.5, 62.5	9.83, 19.7, 32.0
A39426	41048506	50WP	0, 40, 160, 640	0, 40, 80, 160
A39279	41048505	3EC	0, 0, 27, 54, 81	0,0, 9, 12, 18, 36

Following evaluation of the key toxicity endpoints of concern, the NOAEL selected should be based on consistency of effects across studies. Effects noted in most of the studies included clinical symptoms of intoxication and increased incidence of mortality, with the female rat being significantly more sensitive than the male. In selecting the dermal exposure NOAEL, HIARC referred to the two male deaths, in the second 21-day dermal toxicity study (MRID 00146841) using technical endosulfan, which occurred at 9 mg/kg bw/day. HIARC also cited liver histopathological changes starting at 9 mg/kg bw/day. HIARC supported their NOAEL selection by looking at the effect level seen in the EC formulation study (33.3%). *"The toxicity endpoint is supported by another 21-day dermal toxicity study (MRID 41048505) in which clinical signs (tremors, straub-tail, spasms) and mortality occurred in female rats treated dermally with 12 mg/kg of a formulation (33.3% ai) of endosulfan."*[2]

1. Toxicological significance of effects seen at 9 mg/kg/day in the 21-Day Dermal Toxicity Study using Endosulfan Technical (MRID 00146841)

The ETF addressed in detail both the increased incidences of mortality and liver histopathological changes in its response dated January 5, 2001(MRID 45300201). In summary, data from the study clearly show that the males which died had pre-existing non-treatment related developmental deficiencies, and the resulting deaths should not be considered in the overall toxicological evaluation

[2] Ibid., p.22

for endosulfan. Furthermore, no mortalities were seen in male rats at the next highest dose of 27 mg/kg bw/day, and in the other four dermal studies the lowest dose that produced mortality in male rats was 81 mg/kg bw/day. The lack of a clear dose-response within and between studies strongly suggests a non-treatment-related effect. A thorough review of the study also reveals that the liver histopathological findings were considered "very slight" by the pathologist, were only seen in a few animals, and were neither sex- nor dose-related.[3] In addition, a review of the other dermal studies, in some cases with doses significantly higher than in this study, as well as available oral subchronic studies in rats, did not reveal any histopathological changes of significance in the liver. Therefore, the liver effects noted in this study are not of toxicological significance and do not represent a clear adverse effect level.

The ETF does not concur with HIARC's selection of this NOAEL based on either mortality in males or liver histopathology.

2. Effect level in females exposed to endosulfan EC (33.3%) formulation (MRID 41048505)

A review of the 21-day dermal toxicity study using an endosulfan 33.3% EC formulation (MRID 41048505) shows that the female (no. 131) that was found dead from dose group 4 (12 mg/kg/day) was not considered treatment-related. There is no indication from the individual animal data that this female exhibited any clinical or physical signs of toxicity from dermal exposure to endosulfan. Body weight, food consumption and pathological findings were all within normal range. In addition, there was no indication from the report that this animal was ever observed to exhibit clinical neurological signs of toxicity following daily application of the test material. The same results and conclusion, a non-treatment related death, was also made for a female (no. 145) found dead in dose group 5 (18 mg/kg/day). Therefore, the only treatment-related mortalities in females from this study were four animals in the high dose group (36 mg/kg/day).

Based on the information from this report, as well as the other four 21-day dermal toxicity studies, the ETF believes that the most appropriate dermal NOAEL for use in short and intermediate/long-term occupational risk assessment is 12 mg/kg/day.

[3] For a detailed summary of the histopathological findings in this study please refer to Table 3, page 11 of the ETF response "*Volume 1: Toxicology Response by the Endosulfan Task Force to the Health Effects Division Risk Assessment for the Endosulfan Reregistration Eligibility Decision Document Dated February 17, 2000. Selection of a Dermal No Observable Adverse Effect Level (NOAEL).*" MRID 45300201

III. Bioaccumulation potential of endosulfan

A. EPA Conclusion

The HED Risk Assessment Chapter stated the following concerning the potential for endosulfan to bioaccumulate:

"*The 21-day dermal toxicity study in rats (ACC.# 257684/257685) was also selected for Intermediate/long-term dermal exposure. See Short-term Dermal Occupational Exposures above. The dose and endpoint selected for risk assessment is dermal NOAEL= 3 mg/kg/day based on mortality with clinical signs in males, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg/day (LOAEL).*

The 21-day dermal study can also be used for intermediate-/long-term dermal risk assessments because of the appropriateness for the route of exposure and the toxicity is defined and characterized. There is sufficient evidence to believe that endosulfan bioaccumulates with repeated exposure and its toxicity to target organs increases with duration. Endosulfan is structurally similar to other polychlorinated cyclodienes (aldrin, dieldrin, chlordane) which are well known for their toxicities and persistence, slow rate of metabolism, and bioaccumulation in animal tissue. The Committee believes that the severity of the toxicity noted in the 21-day dermal study would increase with duration. This is demonstrated in long term oral studies where the severity and incidence of toxicity (body weight decrease and kidney disease) progresses in the 2-year chronic toxicity study in rats. An MOE of 100 (10x for inter-species extrapolation and 10x for intra-species variability) is generally adequate for occupational exposure. However, in the absence of dermal toxicity studies beyond 30 days exposure, the HIARC requires an additional (FIFRA) factor of 3x to address the uncertainty in extrapolating data from less than 30 days up to several months and/or years, for a total MOE of 300. Since the ETF is not supporting any of the uses that may have resulted in long-term exposures, no long-term exposures are expected.

Intermediate/long-term dermal occupational MOE = 300"[4]

B. ETF Response

(1) Evidence regarding the potential of endosulfan to bioaccumulate

HED made several comments in the risk assessment chapter of the endosulfan RED concerning the potential for endosulfan to bioaccumulate in mammals. Most

[4] Locke D., Endosulfan: HED Risk Assessment for the Endosulfan Reregistration Eligibility Decision (RED) Document. Chemical No. 079401. Case No. 0014. Barcaode D250471. January 31, 2001 (p.22-23)

of these statements were derived from interpretation of two dermal absorption studies (MRID 40223601 and 41048504).

"3.1 Hazard Profile: *Dermal absorption studies in male and female rats showed that endosulfan is slowly absorbed through the skin and slowly excreted, which suggests that endosulfan bioaccumulates in the body."*

"3.6.3 Dermal Absorption: *In reference to the first dermal absorption study (MRID 40223601) – These data showed that significant portions of the dose remained on the skin following soap and water washes. At the 24-hour interval, the data showed endosulfan bioaccumulating in the body of rats."*

"3.6.3 Dermal Absorption: *In reference to the second dermal absorption study (MRID 41048504) – The percent of the dose absorbed at 24 hours was 22.1, 16.1, and 3.8% and at 168 hours was 44.8, 46.4, and 20.3% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The amount of the dose remaining on/in the skin at 168 hours was 41.1, 56.2, and 72.8% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The data showed that endosulfan bioaccumulates in the body of the rats."*

The ETF has reviewed these studies along with repeated dosing toxicokinetic studies in rat and cow. Several key points can be made from the available data on endosulfan:

- A single dose study is not appropriate for determining the potential of a chemical to bioconcentrate. An evaluation of this studies does, however, provide evidence that endosulfan absorbs slowly through the skin, with significant portions of the applied dose remaining in or on the skin at 168 hours.

Table 1: Amounts of Endosulfan (ug) Remaining In/On the Skin over Time (MRID 41048504)

Dose Level	Average Amount of Endosulfan (ug)			
	24 Hours	48 Hours	72 Hours	168 Hours
0.1 mg/kg				
Amount Applied (ug)	20.90	20.70	21.0	21.00
Amount not Penetrated (ug)[1]	17.68	14.50	9.46	8.74
1.0 mg/kg				
Amount Applied (ug)	236.00	237.00	238.00	237.00
Amount not Penetrated (ug)[1]	234.32	193.25	162.00	133.48
10.0 mg/kg				
Amount Applied (ug)	2512.00	2482.00	2510.00	2505.00
Amount not Penetrated (ug)[1]	2381.29	2455.50	2135.19	1824.10

[1]Amount not penetrated = skin wash (ug) + ring extract (ug) + paper cover (ug) + skin at application site (ug)

The data also show that the absorbed dose increases with time reaching a plateau at approximately 48 hours. After 48 hours the percent of absorbed dose excreted increases and the total body burden decreases significantly out to 168 hours, resulting in less than half the residue seen after a single oral dose at 24 hours.

Table 2: Percent of Absorbed Dose of Endosulfan Excreted over Time (MRID 41048504)

Dose Level	Average Amount of Endosulfan (ug)			
	24 hrs	48 hrs	72 hrs	168 hrs
0.1 mg/kg				
Total endosulfan in the animal[1]	2.73	2.62	1.38	0.52
Total Excreted[2]	1.90	4.70	6.79	8.88
Total Penetrated[3]	4.63	7.32	8.16	9.40
% Absorbed Dose Excreted[4]	41.00	64.00	83.00	94.00
1.0 mg/kg				
Total endosulfan in the animal[1]	24.08	36.26	17.62	5.43
Total Excreted[2]	13.86	49.44	53.31	104.78
Total Penetrated[3]	37.94	85.70	70.94	110.20
% Absorbed Dose Excreted[4]	37.00	58.00	75.00	95.00
10.0 mg/kg				
Total endosulfan in the animal[1]	62.46	136.66	91.47	31.99
Total Excreted[2]	33.17	138.98	210.82	476.02
Total Penetrated[3]	95.63	275.63	302.28	508.01
% Absorbed Dose Excreted[4]	35.00	50.00	70.00	94.00

[1]Total in Animal = carcass + liver + kidney + brain + fat + muscle + blood
[2]Total Excreted = urine + feces
[3]Total Penetrated = total in animal + total excreted
[4]% Absorbed Dose Excreted = total excreted/total penetrated x 100

Based on the information provided from this study, once steady state is reached, endosulfan is readily excreted and there is no evidence of bioconcentration in the animal.

- Additional evidence to support the conclusion that endosulfan does not bioconcentrate has been provided in a variety of toxicology and ecological fate studies. A 28-day repeat dosing toxicokinetics study in rats was conducted using ^{14}C-endosulfan (Needham et. al., 1998 - ref. Volume 2). Male and female rats were dosed daily up to 28 days at 1 mg/kg bw/day. The data show that in all tissues the concentration of endosulfan residues increased with repeated dosing, and reached a plateau level during the study. For most tissues this plateau level was reached by the 22nd dose. The maximum residue concentrations were generally low with the exception of the liver and kidney, the organs of metabolism and excretion. Following cessation of dosing the concentration of radioactive residues in all of the tissues fell significantly over the next 5 days to levels similar to those seen 24 hours after a single oral dose.

Table 3: Mean Concentrations of endosulfan residues in tissues following repeated daily oral administration of 1 mg/kg body weight for up to 28 days (Needham et. al., 1998)

Tissue	**Concentration in males (mg equivalents/kg tissue)**							
Day	**2**	**11**	**17**	**23**	**29**	**30**	**32**	**33[(1)]**
Subcutaneous Fat	0.210	0.537	0.604	0.636	0.502	0.443	0.245	0.196
Epididymal fat	0.223	0.634	0.733	0.737	0.546	0.594	0.361	0.213
Plasma	0.114	0.473	0.260	0.404	0.295	0.196	0.130	0.081
Blood	0.157	0.621	0.766	1.211	1.205	1.055	0.942	0.856
Renal fat	0.216	0.516	0.610	0.550	0.461	0.413	0.236	0.129
Liver	0.704	2.234	2.906	4.200	3.368	2.904	1.707	1.683
Kidney	4.118	23.772	29.349	42.668	40.641	38.188	25.422	27.028
Testes	0.047	0.205	0.194	0.244	0.223	0.195	0.157	0.122
	Concentration in females (mg equivalents/kg tissue)							
Day	**2**	**11**	**17**	**23**	**29**	**30**	**32**	**33[(1)]**
Subcutaneous fat	1.114	3.044	2.923	2.962	2.717	1.786	0.904	0.739
Uterus	0.278	0.858	0.296	0.303	1.219	0.305	0.362	0.189
Plasma	0.055	0.234	0.260	0.293	0.268	0.181	0.113	0.071
Blood	0.056	0.301	0.360	0.473	0.505	0.447	0.345	0.263
Renal fat	1.369	3.960	3.230	3.493	3.932	2.815	1.670	1.092
Liver	0.639	3.248	4.898	6.878	5.772	4.623	3.158	3.202
Kidney	1.630	16.818	22.563	31.265	31.616	29.648	19.136	24.720
Ovaries	0.166	0.758	0.746	0.796	0.601	0.651	0.442	0.340

(1) The slight increase in residue levels in kidney and liver of animals sacrificed at day 33 from animals sacrificed at day 32 was most likely due to study design and housing. The animals sacrificed at day 32 had been in metabolism cages (metabowls) for 4 days and, due to the design of the cages, ate less and drank more than the animals housed in standard holding cages. This change lead to an increase in urinary clearance of polar metabolites, resulting in slightly less residues in liver and kidney.

Following normalization of the excretion results (Table 4) it can be seen that 12.7±1.7% of the excreted radioactivity was found in the urine and 65.5±3.5% in the feces. Overall the results for the excretion balance study showed that the route and rate of excretion of endosulfan residues was not significantly altered following multiple oral dosing as compared to following a single oral dose.

Table 4: Excretion of radiolabeled dose, as a percentage of recovered radioactivity, from rats following the last of 28 daily oral doses of 1 mg/kg bodyweight.

Sample	**Excretion of radioactivity (as % of recovered radioactivity)**								
	Males					**Females**			
	041M	**042M**	**043M**	**044M**		**045F**	**046F**	**047F**	**048F**
Urine									
Total after 96 hrs	10.83	12.30	10.83	12.44		13.30	11.56	14.98	15.03
Fecal organic extract									
Total after 96 hrs	3.95	3.47	3.16	5.33		3.24	7.48	2.14	3.55
Fecal aqueous residue (non-organic extractable)									
Total after 96 hrs	65.74	60.97	62.22	64.47		62.33	60.16	58.65	57.19
Cage wash									
Total after 96 hrs	2.16	2.01	2.82	1.50		3.39	3.36	4.20	2.90
Carcass	6.55	7.19	7.18	5.70		5.97	7.22	8.73	8.41
Tissues	10.77	14.06	13.81	10.62		11.77	10.23	11.32	12.92
Overall Total	100.00	100.03	100.02	100.06		100.00	100.00	100.01	100.00

Similar findings were reported in another repeat dose study in rats by Dorough et. al., 1978 (MRID 05003703). As part of this study two groups of female rats were fed diets containing 5 ppm of either α-endosulfan or β-endosulfan for up to 14 days, followed by a 14-day depuration period. As with the above mentioned study, endosulfan residues increased in the kidney and liver following repeated administration, but showed no accumulation in fat, muscle or brain, and decreased significantly following cessation of dosing.

Table 5: Residues in tissues of female rats fed 5 ppm of α- or β-[^{14}C] endosulfan in the diet (MRID 05003703)

	Parts per million [^{14}C]endosulfan equivalents per isomer in diet									
	Kidney		Liver		Visceral fat		Subcutaneous fat		Muscle	Brain
Days	α	β	α	β	α	β	α	β	α/β	α/β
On treatment										
1	0.38	0.47	0.26	0.32	0.34	0.24	0.32	0.30	0.02	0.03
2	1.26	1.21	1.02	0.79	0.85	1.02	0.23	0.34	0.02	0.03
7	1.77	1.87	0.96	0.75	0.74	0.53	0.51	0.30	0.02	0.04
10	2.28	2.08	1.11	0.94	0.94	0.55	0.15	0.28	0.03	0.04
14	3.00	3.26	1.08	1.06	0.62	0.50	0.15	0.32	0.05	0.07
Off treatment										
1	2.75	3.34	1.00	0.87	0.45	0.42	0.02	0.08	0.05	0.05
3	1.89	2.21	0.49	0.57	0.15	0.28	<0.02	<0.02	0.02	0.06
7	1.53	1.66	0.28	0.36	<0.02	<0.02	<0.02	<0.02	<0.02	0.04
14	0.94	0.92	0.11	0.19	<0.02	<0.02	<0.02	<0.02	<0.02	0.02

In addition to the 28-day rat study, there is a 28-day feeding study in lactating cows (MRID 44843702). In this study residues of α-endosulfan, β-endosulfan and endosulfan sulfate were measured in milk and edible tissues following dosing at nominal feeding levels of 0, 4, 12, and 30 ppm. Based on the average daily intake of feed the nominal intake of endosulfan was 0, 80, 240 and 600 mg/cow/day. For all residue samples analyzed, the values for α-endosulfan and β-endosulfan were mostly below the respective LOQs. Endosulfan sulfate accounted for the majority of residue, and feed-to-tissue transfer factors were relatively low.

Table 6: Endosulfan sulfate residues following 28 days of dosing in lactating cows (MRID 44843702)

Substrate	Nominal dose level (ppm)	Number of days dosing	Mean Endosulfan sulfate (mg/kg)	Transfer Factor
Whole Milk	4	10-28	0.07	0.02
	12	10-28	0.27	0.02
	30	10-28	0.62	0.02
Muscle	4	28	0.04	0.01
	12	28	0.21	0.02
	30	28	0.76	0.03

Substrate	Nominal dose level (ppm)	Number of days dosing	Mean Endosulfan sulfate (mg/kg)	Transfer Factor
Liver	4	28	0.71	0.18
	12	28	2.00	0.17
	30	28	3.20	0.11
Kidney	4	28	0.07	0.02
	12	28	0.31	0.03
	30	28	0.67	0.02
Fat	4	28	1.40	0.35
	12	28	4.70	0.39
	30	28	9.90	0.33

As seen in the rat toxicokinetic study, endosulfan sulfate residues increased in the milk with continued dosing until day 10, when the levels reached a plateau at the high and mid doses for the remaining 18 days of dosing. A plateau level in the low dose group was reached within the first 4 days of dosing, with no change in residues for the remainder of the dosing period.

Table 7: Mean endosulfan sulfate residues in whole milk (MRID 44843702)

Number of days dosing	Mean endosulfan sulfate residues (mg/kg)		
	Cows 4, 5 &6 4 ppm diet	Cows 7, 8 & 9 12 ppm diet	Cows 10, 11 & 12 30 ppm diet
-1	Not Detected	Not Detected	Not Detected
1	<0.01	0.02	0.04
4	0.07	0.20	0.53
7	0.06	0.23	0.50
9	-	0.23	-
10	0.07	0.27	0.56
13	0.07	0.27	0.61
16	0.07	0.24	0.62
19	0.07	0.26	0.68
22	0.06	0.24	0.64
25	0.07	0.31	0.56
28	0.06	0.28	0.66

Also similar to the results from the rat toxicokinetic study, depuration of up to 21 days resulted in significant decreases in endosulfan sulfate residues. Endosulfan sulfate residues in milk followed a steady exponential decline, with an initial half-life within the first four days.

Table 8: Reduction of endosulfan sulfate residues in cow tissue following 21 days of depuration (MRID 44843702)

Number of days depuration	Cow Numbers	Mean endosulfan sulfate residue (mg/kg)			
		Muscle	liver	Kidney	fat
0[(1)]	10, 11 &12	0.76	3.20	0.67	9.9
7	13	0.06	0.76	0.10	5.1
14	14	0.04	0.54	0.07	2.1
21	15 &16	0.03	0.36	0.06	1.1

[(1)] Mean data from animals in top dose group (28 days dosing)

Table 9: Reduction of endosulfan sulfate residues in milk following 21 days of depuration (MRID 44843702)

Number of days depuration	Cow numbers	Mean endosulfan sulfate milk residue (mg/kg)
-2[(1)]	13, 14, 15 & 16	0.94
1	13, 14, 15 & 16	0.69
4	13, 14, 15 & 16	0.18
7	13, 14, 15 & 16	0.09
10	14, 15 & 16	0.15
13	14, 15 & 16	0.09
16	15 & 16	0.05
19	15 & 16	0.06
22[(2)]	15 & 16	0.04

[(1)]equal to 27 days dosing; [(2)] morning milking only

Lastly, analysis of livers and kidneys from rats and mice administered endosulfan for two years showed extremely low residues of endosulfan sulfate. A two-year feeding study was conducted in rats (MRID 41229001) at dose levels of 0, 3, 7.5, 15 and 75 mg/kg/day. After completion of feeding, the animals were sacrificed and analyses were carried out to determine the levels of endosulfan and its main metabolites endosulfan-hydroxyether, -sulfate, -lactone and –diol in liver and kidney. As no residues above the limits of quantification (LOQ 0.10 – 0.12) were observed in livers and kidneys of the 15 mg/kg/day animals, the organs of the two lower doses were not investigated further. Neither α-endosulfan nor β-endosulfan, which were fed in the diet, was observed above the LOQ in any animals. The only metabolite detected above the LOQ was endosulfan sulfate, which was found in concentrations of 0.2 – 0.4 mg/kg in the livers of animals dosed at 75 mg/kg/day. No residues were observed in the kidneys of the 75 mg/kg/day dose group animals. A two-year feeding study in mice (MRID 41229002) was conducted at dose levels of 0, 2, 6 and 18 mg/kg/day in the diet. At the end of the two-year feeding study the livers and kidneys of the mice were analyzed for endosulfan and its main metabolites. The compounds α-endosulfan and β-endosulfan could not be detected in any tissue samples. The levels of the metabolites endosulfan-hydroxyether, -lactone and –diol were below or just above the LOQ (0.02 mg/kg). Endosulfan-sulfate was found in the kidneys at 0.1 – 0.2 mg/kg/day and in the livers at 0.7 – 1.1 mg/kg in the high dose animals. In neither study was there any indication of bioconcentration in the target organs.

Information from the above mentioned studies clearly defines the kinetics of endosulfan following oral and dermal routes of administration. While there is an indication that endosulfan increases in tissue concentration following repeated exposures, the data also show that tissue levels plateau within 2 to 21 days depending on the route of exposure. In addition, mammalian and aquatic organism have shown that any cessation of exposure results in rapid and significant reductions in body burden of endosulfan. Therefore, endosulfan would not be expected to bioconcentrate in workers over intermediate/long-term exposures.

(2) Fate of Endosulfan compared to other polychlorinated cyclodienes

While endosulfan may be structurally similar to other polychlorinated cyclodienes, its behavior with respect to bioconcentration and bioaccumulation is distinctly different. Studies in aquatic and terrestrial systems have shown rapid decline of endosulfan residues during depuration, with half-lives ranging from hours to a few weeks. A 1993 review by the Agency for Toxic Substances and Disease Registry (ATSDR) concluded, "endosulfan does not bioaccumulate to high concentrations in terrestrial or aquatic ecosystems."[5] Other sources in the public literature have also made the same conclusions regarding the potential for endosulfan to bioaccumulate in the environment based on detailed reviews of available laboratory and field data.[6,7] The EFED Risk Assessment for the RED on endosulfan, dated April 13, 2001, also concluded "Based on the available data, it appears that endosulfan is not likely to be strongly bioaccumulative." (p. 32)

(3) Severity and incidence of toxicity from subchronic to chronic exposure

Cumulative toxicity is defined as "progressive injury produced by summation of incremental injury resulting from successive exposures."[8] Examples of cumulative toxicity include liver fibrous from ethanol ingestion and squamous cell metaplasia from repeated dermal exposure to formaldehyde. Based on this interpretation of cumulative toxicity, the ETF reviewed the appropriate subchronic and chronic studies for endosulfan in order to address EPA's concerns, and has concluded that endosulfan is not cumulatively toxic.

The NOAEL from the subchronic feeding study in rats (MRID 00145668) of 0.5 mg/kg/day and the NOAEL from the two-year chronic study in rats (MRID 41099502) of 0.6 mg/kg/day are essentially identical, as are the LOAELs of 1.5 and 2.9 mg/kg/day, respectively. While both LOAELs correspond to changes in the kidneys, there is no clear progressive or incremental injury identified. Effects reported in the subchronic studies were limited to physiological adaptive changes, while the effect noted in the chronic study was associated with a slight increase in a commonly occurring legion in aging rats.

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[5] U.S. Department of Health & Human Services, Public Health Services, Agency for Toxic Substances and Disease Registry. "Toxicological Profile for Endosulfan," TP-91/16; PB 93-182558. April 1993. (pp. 124)
[6] World Health Organization (1984) "Environmental Health Criteria 40: Endosulfan." IPCS International Programme on Chemical Safety. (p.10)
[7] Navqvi S.M., and Vaishnavi C., (1993) "Bioaccumulation Potential and Toxicity of Endosulfan Insecticide to Non-Target Animals." Comp. Biochem. Physiol. Vol. 105C, No. 3, pp.347-361.
[8] Ballantyne B., Marrs T.C., and Syversen T., (2000) *Fundamentals of Toxicology.* General Applied Toxicology, Macmillan Reference LTD, London UK, p. 6.

There are two subchronic feeding studies conducted in the rat using endosulfan technical. The first study was a 13-week feeding study with a 4-week recovery period (MRID 00145668). Kidney effects in this study were restricted to granular pigmentation and lysosomal storage of metabolized endosulfan in the proximal convoluted tubules of the kidney. This physiological effect resulting from metabolic activity in the tubules did not cause any histopathological changes to the kidneys. There was an additional notation by HED in the review of this study regarding an increased incidence of basophils in the cortical area of the tubules. This effect is considered an adaptive response to the accumulation of endosulfan metabolite residues in the lysosomes in the tubules and should not be considered a "kidney abnormality". A second study (MRID 40767601), 30-day feeding, was conducted in the rat to further investigate by light and electron microscopy the pigmentation effects noted in the 13-week study. This investigation confirmed that the lysosomal changes represented storage and metabolism of endosulfan in the kidney. The study also showed that storage of endosulfan metabolites in the kidney is reversible and did not result in any cytotoxic effects. Negative Prussian blue reaction in both the liver and kidney confirmed a lack of hemosiderosis, disproving an initial assumption made in the original EPA review (MRID 41775501).

In the two-year chronic study in rats (MRID 41099502) the primary kidney effect was progressive glomerulonephrosis (PGN), a commonly found lesion in aging rats. In the review by HED, the Agency concluded "the increase in the severity of progressive glomerulonephrosis in rats of both sexes at the high-dose level was regarded as an adverse effect and that the spontaneously occurring renal disease was exacerbated by exposure to the test material." While EPA considered this an adverse effect, the incidence in the high dose males (43%) was not significantly different from the historical control range (10-38%) and the average age of animals effected was 96 weeks, clearly showing an age-dependent effect. In addition, the incidence of cortical areas of basophilia in the tubules was lower than controls (7 vs. 10%), and there were no other indications of histopathological changes in the proximal tubules (which would have been expected if a cumulative or progressive toxic insult was occurring). Lastly, as stated previously, analyses of the liver and kidneys of rats after a two-year exposure to endosulfan showed no bioconcentration of residues in either target organ.

There is no evidence provided in either the subchronic or chronic studies to support EPA's suggestion that endosulfan bioaccumulates or that longer-term exposure would result in cumulative effects.

(4) Requirement for an additional FIFRA uncertainty factor for assessment of intermediate/long-term occupational risk assessment

There is no evidence from the available data that endosulfan would be expected to bioconcentrate in workers following an intermediate or long-term exposure period. Nor is there any evidence to support EPA's supposition that longer-term dermal exposure would result in increased toxicity. Dermal absorption studies with endosulfan clearly show that endosulfan is absorbed very slowly through the skin, and once the skin is penetrated and a steady-state is reached, metabolism and excretion is rapid and complete. Repeated dermal exposures longer than 30-days would result in a plateau of residues in the body within 2 to 21 days, and any cessation of exposure would result in significant reductions in body burden. Whether exposure is intermediate or long-term, data show that the 30-day dermal toxicity studies are adequate for assessment of risk to workers, and no additional uncertainty factor is required.

IV. Conclusion

Based on the information provided, the ETF believes that the appropriate dermal NOAEL for short-term and intermediate/long-term occupational exposure is 12 mg/kg bw/day, based on increased incidence of mortality in females at 27 mg/kg bw/day. The ETF also concludes from the above data evaluation that endosulfan does not bioconcentrate, and that repeated dermal exposure to endosulfan will not result in increased toxicity from intermediate or long-term occupational exposures. Therefore, there is no requirement for an additional FIFRA uncertainty factor for intermediate or long-term occupational risk assessments.

APPENDIX 1

Ebert, E.; Leist, K.-H.; Kramer, M. (1985a) Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in SPF Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A30754; Date: 11 Mar 1985a (MRID 00147744)

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), OECD Test Guideline 410 (May 1981) and EPA-FIFRA §82-2 (November 1982)
Material and Methods : Groups of 11 male and 11 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan (purity 97.2%) in sesame oil for 6 hr/day under total occlusion for 30 days, 5 days/week, in total 21 treatments, at doses of 0, 3, 6, 12, 48, 96 or 192 mg/kg bw. The two low doses were given to females only. The 2 high doses were given to males only. After the 6 hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded twice daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. On day 31 of the study 6 rats/group/sex were sacrificed, while the remaining animals were allowed a recovery period of 14 days. After sacrifice standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology were carried out.
Findings: This study should be evaluated in conjunction with the described above follow-up study. Main findings are summarized in Table A1.
Mortality: Mortalities occurred between day 2 - 22. At 3, 6 and 12 mg/kg bw/day one female died. These deaths were attributed to the application technique employed and for this reason a follow-up study was started (see above). At 48 mg/kg bw/day 4/11 females died on day 2, 4 or 22. At 192 mg/kg, 2/11 males died due to substance related signs (tremors, hypersalivation and tonic-clonic convulsions).
Clinical signs: No clinical signs were seen in any of the males at 96 mg/kg bw/day. At the high dose, tremors and salivation were observed in 3/11 males, two of which died on day 6 and 9. In the females, first clinical signs started at 12 mg/kg bw/day and consisted of pilo-erection, increased salivation and lacrimation. At higher doses blood encrusted snouts and chromodacryorrhoea were observed. In addition, at the highest dose for the female rats (48 mg/kg bw/day) tonic-clonic convulsions were observed in one female.
Irritation: Dryness and desquamation were seen after the 4th application. These signs had reversed after 3 days.
Body weights: No effects were found on body weights or body weight gain.
Food consumption: No differences were observed on food consumption between the groups.
Water consumption: No differences were observed in water consumption between the groups.
Hematology: No statistically significant hematological effects were found.

Clinical chemistry: Various slight changes were observed. None of these was dose related: Cholinesterase activity was reduced in serum of males at 192 ppm. No effect on serum-, erythrocyte-, or brain ChE-activity was seen after the recovery period.
Other parameters: No changes were found in urinalysis, organ weights, or macroscopic pathology or histology.

Table A1: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex		Males					Females				
Dose	(mg/kg bw/day)	0	12	48	96	192	0	3	6	12	48
Mortalities		0	0	0	0	2	0	1[1]	1[1]	1[1]	4
Hypersalivation		-	-	-	-	3	-	-	-	-	4
Tremors/Convulsions		-	-	-	-	1	-	-	-	-	1
Autoagression[1]		-	-	-	-	-	-	1	1	1	-
Erythrocyte ChE	(U/L)	247	215	178*	237	218	217	207	205	202	223
Serum ChE	(U/L)	428	374	378	381	288*	1298	1209	1193	1339	1022
Brain ChE	(U/kg)	5592	5543	5570	4666*	5198	5190	4875	4521	4972	5189

[1] The self inflicted wounds and subsequent deaths were caused by biting in order to remove the tight bandage around the trunk.

Conclusions: In this 30-day dermal study with 14-day recovery the LOAEL was 48 mg/kg bw/day based on female deaths. Other female deaths, seen at 3, 6 and 12 mg/kg bw/day, are not considered substance related, but was caused by the tight occlusive bandage resulting in self-inflicted wounds and deaths. As a result of these findings the study was repeated with more comfortable bandaging and, based on the results of this study, with adjusted doses. Essentially no cholinesterase inhibition was found in erythrocytes, serum or brain.

APPENDIX 2

Ebert, E., Leist, K.-H., Kramer, M.(1985b) Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A30753; Date: 22 Feb. 1985 (MRID 00146841)

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Material and Methods: Groups of 6 male and 6 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan (purity 97.2%) in sesame oil for 6 hr/day under total occlusion for 30 days, 5 days/week, in total 21 treatments, at doses of 0, 1, 3, 9, 27 mg/kg bw or at 81 mg/kg bw (males only). After the 6-hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded twice daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. At day 31 the animals were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out.
Findings: The main findings are summarized in Table A2. This follow-up study should be evaluated in combination with the first study, described below.
Mortality: Mortalities occurred between day 2 - 8. The two male deaths at 9 mg/kg cannot be considered as treatment related due to developmental disturbances originating prior to treatment: Both males had small immature testes and one also had a very small liver, while the liver weight of the other could not be determined due to autolysis. The developmental disturbance in these animals is likely to have reduced the capacity to metabolize the chemical. No male deaths were observed at 27 mg/kg bw/day.
In addition, 5 females at 27 mg/kg died between day 2 and 6 without any signs of toxicity.
Clinical signs: No clinical signs were seen in any female. One of the 3 males that died at the top dose (81 mg/kg) showed signs of typical of endosulfan intoxication: tono-clonic convulsions, increased salivation and increased respiratory rate.
Irritation: At the start of the treatment a slight irritation was seen at all dose levels and the control group. This subsided within a few days.
Body weights: No effects were found on body weights or body weight gain.
Food consumption: There was a marked reduction in food intake in animals that died inter-currently.
Water consumption: There was a marked reduction in water intake in animals that died inter-currently.
Hematology: No hematological effects were found. The slight reduction in thrombocytes, though statistically significant, falls still within the normal range.
Clinical chemistry: Cholinesterase activity was reduced in serum of males treated at 9 ppm and above. This effect is not judged to be of toxicological relevance: The reduction

was not found in the females, which are more sensitive for endosulfan toxicity. Furthermore, this effect did not occur in the first 30-day dermal toxicity study, though higher doses were applied there. In addition, an *in vitro* assay is available indicating that endosulfan is not a primary AChE-inhibitor. In subchronic and chronic feeding studies on endosulfan there was no evidence of cholinesterase inhibition independent of the species tested (mouse, rat or dog). In addition, in workers producing or applying endosulfan, cholinesterase inhibition has never been found. There is consistency in both dermal subchronic studies about the absence of erythrocyte acetyl-cholinesterase. Therefore, the serum cholinesterase inhibition found only in the second study must have been a spontaneous finding. This effect is not evaluated by WHO as an adverse effect. The change in brain cholinesterase activity was within the normal variation for rats of this age, was not dose-related, and was not consistent with other endosulfan dermal studies. All other serum clinical chemical parameters were also within normal range
Organ weights: All organ weights, including spleen weights with a slight statistical difference in females only, were within a normal range of biological variation.
Urinalysis: No changes were found.
Macroscopic pathology or histology: No macroscopic changes were observed at necropsy. About the microscopic changes the original report states: "The livers showed enlargement of parenchymal cells in peripheral sectors, together with loss of cytoplasmic basophilia; there were also isolated cell necroses and frequent mitoses. These changes occurred from 9 mg/kg onwards, but were encountered only in a few animals and only to a very slight extent; they did not increase with higher doses and were not sex-related." The changes were slight and infrequent. Therefore it is doubtful how these effects are related to the test substance and whether they are toxicologically relevant. These slight effects in the liver were not found in the first study using higher doses. A third study, though in adequate in some aspects, also reported absence of histological effects (**Dikshith et al. 1988**).

Table A2: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex/Group size		Males/ 6						Females/ 6				
Dose (mg/kg bw/day)		0	1	3	9	27	81	0	1	3	9	27[1]
Mortalities		0	0	0	2	0	3	0	0	0	0	5
Spleen weights	(g)	0.50	0.58	0.55	0.41	0.53	0.52	0.36	0.43	0.41	0.46*	0.44
	(% bw)	0.17	0.19	0.18	0.16	0.18	0.18	0.17	0.20	0.19	0.21*	0.19
Erythrocyte ChE	(U/l)	209	222	206	222	218	218	192	180	180	202	181
Serum ChE	(U/l)	348	306	296	97*	96*	72*	472	436	381	291	285
Brain ChE	(U/kg)	4438	4153	3802*	3490*	3181*	3357*	3552	3056*	2916*	3065*	3174

[1] Due to one survivor only it was not possible to establish statistical significance in this group.

Conclusions: The LOAEL of 27 mg/kg bw/day for female rats and of 81 mg/kg bw/day for male rats in this 30-day dermal study are based on death observed at these doses. The slight serum cholinesterase inhibition in the males is not considered to be of toxicological significance. From sensitive *in vitro* data it is well known that endosulfan is not a cholinesterase inhibitor. The slight, non-dose-related, brain cholinesterase inhibition in

males and females is probably not substance related, since no corresponding changes were found in pharmacologically closely related erythrocyte cholinesterase activity. Moreover, these changes were not found in another subchronic dermal study using higher dermal doses. Therefore these changes must be considered as a spontaneous finding. Two male deaths at 9 mg/kg cannot be considered as treatment related due to developmental disturbances originating prior to treatment. Therefore, the NOAEL for female rats in this study was 9 mg/kg and for male rats between 27 and 81 mg/kg bw/day.

APPENDIX 3

Dikshith, T.S.S.; Raizada, R.B.; Kumar, S.N. (1988); Effect of repeated dermal application of Endosulfan to rats; Generated by: Ind. Toxicol. Res. Cent., India; Vet. Human Toxicol. Vol. 30, 219 - 224 (1988); Company file No: A41365; Date: 15 Feb. 1988

GLP: The publication was not conducted according to GLP.
Guidelines: The publication does not state that the study was carried in compliance with any guideline.
Information on the methods is scanty and results are sometimes inconsistent. Therefore, the study is **inadequate**.
Materials and Methods: Groups of 6 male and 6 female Wistar rats were painted daily with dermal applications of endosulfan (origin unspecified) in acetone to the shaved lateral abdominal skin for 30 days. Males were dosed at 18.75, 37.50, 62.50 mg/kg bw/day, females at doses of 9.83, 19.66, 32.00 mg/kg. A control group was skin painted with peanut oil. After this period animals were killed by decapitation. At necropsy organs were weighed, enzyme activities in liver and serum measured, and standard hematology was conducted. Macroscopic and histopathological examination was carried out on liver, kidneys, spleen, brain, testes, epididymis, adrenals, ovary and cervix. Residue analysis was carried out in liver, kidney, testes, brain, fat and blood.
Findings:
Mortality: None of the dosed animals died.
Clinical signs: Hyper excitation, tremors, dyspnea and salivation were seen initially. These signs had resolved within one week. The doses at which these signs were observed were not specified.
Body and organ weights: No changes were observed in body weights or relative organ weights.
Hematology: No significant hematological changes were found in female rats. Inconsistent changes were found in the males. Hemoglobin was slightly reduced in all male groups. The number of leukocytes was increased at 62.5 mg/kg and decreased at 37.5 mg/kg. At the latter dose the percentage of lymphocytes had increased and neutrophils reduced. These effects can be dismissed due to the minimal size of the changes or the lack of dose relationship.
Biochemistry: Liver serum GPT activities were significantly decreased. Liver GOT was also significantly decreased but serum GOT was increased. These changes were not dose-related. Various other inconsistent changes were measured.

Table A3: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex	Males				Females			
Dose (mg/kg bw/day)	0	18.75	37.5	62.5	0	9.83	19.66	32.0
Hemoglobin (g/100 ml)	16.9	16.2*	15.6*	15.8*	16.5	17.0	17.0	16.0
White Bl. Cells ($10^3/mm^3$)	7775	7025	6900*	8425*	8080	8145	8100	8000
Lymphocytes (%)	76	75	86*	76	83	85	86	81
Neutrophils (%)	23	24	13*	22	17	13	12	15
Liver GOT (µmol/g/min)	14.8	9.7*	5.6*	9.1*	8.4	5.4*	6.7	5.4*
Liver GPT (µmol/g/min)	37.0	14.8*	15.2*	14.7*	21.6	14.8*	15.1*	11.2*
Liver Protein(mg/g tissue)	138	123	109*	117*	152	162	186*	195*
Serum GOT (nmol/g/min)	55	72*	69*	59	38	27	54	84*
Serum GPT (nmol/g/min)	102	77*	32**	83	86	47*	67*	49*
Serum AP (nmol/g/min)	396	776*	1095**	730*	505	639	567	539
Serum Protein (mg/ml)	105	109	118	107	85	101*	105*	117**

Histology: No gross or microscopic abnormalities were found in the major organs.
Residue analysis: Residue levels in organs of males in decreasing order were: fat, kidneys, testes, blood, liver, brain; and in organs of the females: fat, liver, blood, kidneys, brain. Residues in females were much higher than in males. Details about the method are not given and the results are inconsistent with the other metabolism studies.
Conclusion: In this 30-day dermal study endosulfan caused transient neurological effects only in the first week. This points to adaptation e.g. by induction of detoxifying enzymes. Biochemical parameters in liver and serum were changed in a non-dose related fashion. In absence of significant biological effects (including histological effects) the NOAEL was probably equal to the highest doses applied.

APPENDIX 4

Thevenaz, Ph.; Luetkemeier, H.; Chevalier, H.J.; Vogel, W.; and Terrier, Ch. (1988); Endosulfan – Emusifiable Concentrate (Code: HOE 002671 OI EC34 A101). Subchronic (4-Week) Repeated Dose Dermal Toxicity Study in Rats; Research & Consulting Co. AG, Switzerland; Company file No: A39279; Date: 4 Oct. 1988; EPA MRID 41048505

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Materials and Methods: Groups of 15 male and 15 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan emulsifiable concentrate (33.3% technical in carboxymethylcellulose) for 6 hours/day, 5 days/week for a total of 21-22 applications. There were two control groups, the first received vehicle alone (aqueous 4% carboxymethylcellulose solution), and the second group received formulation base (HOE 002671 OI EC00 A302, administered in vehicle). Male rats were dosed 27, 54 and 81mg/kg bw/day, females at doses of 9, 12, 18 and 36 mg/kg bw/day. After the 6-hour treatment period the bandage was removed and the treated skin was rinse with lukewarm water and dried with a disposable paper towel. Mortality was recorded twice daily and clinical signs were recorded at least once daily. Examination of the treated skin area was done before the next application, food consumption and body weights were recorded weekly. At day 31 the animals were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out.

Findings:
Mortality: One female (no. 131) at 12 mg/kg bw/day and one female (no. 145) at 18 mg/kg bw/day were found dead during treatment week 4. Four females (nos. 151, 152, 160 and 165) at 36 mg/kg bw/day were found dead during treatment weeks 1, 2 and 4. The incidence of mortality in females in the high dose group was considered treatment-related, though no pathomorphological lesions could be distinctly attributed to administration of the test article. One male (no. 11) from vehicle control died following anesthesia for blood sampling during week 3 of recovery period. One female (no. 146) at 18 mg/kg bw/day died following blood sampling during treatment week 4. Neither of these deaths was attributed to treatment.
Clinical signs: Transient signs ranging from slight to severe in intensity were observed after the end of the application period: tremor, Straub-tail, trismus, saltatory spasms, extension spasms, tetanoid spasms. Onset occurred within one hour after daily application, the duration did not exceed approximately 30 minutes. Occurrence was limited to males in the 81 mg/kg bw/day dose group and females in the 18 and 36 mg/kg bw/day dose groups, with isolated cases recorded in females at 12 mg/kg bw/day. One female in the 36 mg/kg bw/day dose group died following one spasm attack. No comparable signs were observed in during the recovery period.

Irritation: In the formulation base control group, erythema of minimal to moderate intensity was observed during both treatment and recovery periods. Mean erythema values increased towards the end of the treatment period, with concomitant slight edema from week 3 of the study. Edema was not detectable from week 2 of recovery on, and erythema decreased from moderate to marginal mean values over the duration of the treatment-free period. During the second half of the treatment period, marginal to slight erythema was observed in dosage groups 3 to 6, with concomitant marginal edema occurring in groups 4 and 5. During the recovery period, marginal erythema persisted during week 1, whereas edema was no longer detectable. No effects were noted in the vehicle control group.
Body and organ weights: No changes were observed in body weights or relative organ weights.
Hematology: No toxicologically significant hematological changes were found in male or female rats.
Biochemistry: Slightly increased levels of alkaline phosphatase activity a decrease in albumin to globulin ratio was seen in high dose females. Slightly increased aspartate aminotransferase activity was noted in the high dose males. Slightly decreased albumin concentrations were seen in the 18 and 36 mg/kg bw/day dose groups for female rats. Plasma cholinesterase activity was slight decreased (by 22-32%) for females at 12, 18 and 36 mg/kg bw/day.
Histology: Other than skin effects resulting from irritation, no gross or microscopic abnormalities were found in the major organs.

Table A4: Endosulfan EC33; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex	Males					Females					
Dose (mg/kg bw/day)	0	0	27	54	81	0	0	9	12	18	36
Mortalities	1[1]	0	0	0	0	0	0	0	1	2[1]	4
Erythrocyte ChE (umol-SH/ml)[2]	1.89	1.85	1.64*	1.56*	1.48*	2.02	1.87	1.92	1.76*	1.82	1.69*
Serum ChE (umol-SH/ml)[2]	0.66	0.68	0.70	0.72	0.70	3.07	2.58	2.56	2.40*	2.19*	2.08*
Brain ChE (umol-SH/g)[2]	5.98	6.13	6.02	5.03*	6.64*	7.63	6.91*	7.32	6.73*	6.95*	6.65*

[1] Non-treatement-related, deaths occurred during blood sampling
[2]Values taken from end of treatment. There were no significant changes following the 4-week recovery period in any dose group.

Conclusion: In this 30-day dermal study endosulfan caused transient neurological effects following each treatment period in the high dose group animals. The NOAEL for systemic toxicity was considered 54 mg/kg bw/day for males and 9 mg/kg bw/day for females. A decrease in erythrocyte cholinesterase was slight, but dose-related in males only at all dose levels.

APPENDIX 5

Ebert, E. (1987) Endosulfan – Water Dispersible Powder (50%) (Code: Hoe 002671 0I WP50 A501) Subchronic Dermal Toxicity (21 Applications in 30 Days) in Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A39426; Date: 17 May 1987. EPA MRID 41048506

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Material and Methods: Groups of 11 male and 11 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan WP (purity 49.5%) in the form of 2 – 32% aqueous dispersions under total occlusion 6 hours/day, 5 days/week (21 treatments in total) for 30 days. Dosages were 0, 40, 80 (females only), 160 and 640 mg/kg bw/day (males only). After the 6-hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. At day 31 the animals in the main group (6/sex/dose) were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out. A recovery group of 5/sex/dose were sacrificed on days 23 or 24 after termination of the treatment.
Findings: The main findings are summarized in Table A.
Mortality: Mortalities occurred between day 2 – 24 in the females only. Three animals in the highest dose group (160 mg/kg bw/day) dies on day 3, 11 and 24, respectively. None of these animals showed previous clinical signs of intoxication. One female in the 80 mg/kg bw/day group died day 21.
Clinical signs: One female in the 80 mg/kg bw/day group showed dacryohemorrhea and a blood-crusted snout on day 22 of the study. No clinical signs of intoxication or mortality were noted at any dose in the male rats. No signs of neurological disturbance, changes in the eyes, damaged to the oral mucosa or impairment of dental growth were observed in any of the treatment groups.
Irritation: At the end of the first and during the second week of treatment, there was a slight redness of the skin in individual animals from the 80 mg group and in many of the animals from the 160/640 mg groups. Dry and chapped skin was noted in the high dose animals with fine or course scales. These signs of irritation receded by the end of the second week, and were only present in a few individual animals during the third week of the study.
Body weights: From the second week of treatment onwards, males in the 640 mg/kg bw/day group showed significantly reduced body weight gains when compared to controls. No other dose group showed significant weight changes.
Food consumption: There was a marked reduction in food intake
Water consumption: There was a marked reduction in water intake
Hematology: No hematological effects were found.

Clinical chemistry: Cholinesterase activity was reduced in serum of females treated at 80 and 160 mg/kg bw/day. This effect, which was discernible as a tendency after the end of the recovery period, was possibly due to reduced biosynthesis of the enzyme in the liver and could be interpreted as an impairment of hepatic function. There were no indication s of cholinesterase inhibition, since a comparable effect of the substance on erythrocytes and brain ChE was not observed. There was also a slight increase in cholesterol and total lipids in the high dose females. All other serum clinical chemical parameters were also within normal range
Organ weights: All organ weights were within a normal range of biological variation.
Urinalysis: No changes were found.
Macroscopic pathology or histology: No macroscopic or microscopic changes were observed at necropsy.

Table A5: Endosulfan 50WP; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex/Group size		Males/6				Females/6			
Dose (mg/kg bw/day)		0	40	160	640	0	40	80	160
Mortalities		0	0	0	0	0	0	1	3
Erythrocyte ChE	(U/l)	332	424	356	379	514	507	486	548
Serum ChE	(U/l)	467	407*	426	405*	1318	1005	952*	709*
Brain ChE	(U/kg)	4267	4538	4579	4891*	4173	4257	3944	4074

Conclusions: The LOAEL of 80 mg/kg bw/day for female rats and of 160 mg/kg bw/day for male rats in this 30-day dermal study are based on death observed at these doses. The slight serum cholinesterase inhibition in the females is not considered to be of toxicological significance. From sensitive *in vitro* data it is well known that endosulfan is not a cholinesterase inhibitor.

Appendix 6

CONSIDERATION OF SKIN PENETRATION FOR RISK ASSESSMENT IN MAN

For risk assessment in man the dermal absorption (percent) for a 8-10 hour exposure period is normally selected since it reflects an average working day for the pesticide handlers (mixer/loader/applicator). In addition, interspecies differences (experimental animal to man) in skin penetration have to taken into consideration

In vivo dermal penetration study in the rat

For extrapolation of animal data to the human situation, an *in vivo* dermal penetration study in the rat has been conducted (MRID 41048504).
Groups of 16 female Sprague Dawley rats were exposed dermally for 10 hours to a similar-to-field-use EC33 formulation of ^{14}C-endosulfan on a circular area of shorn dorsal skin at actual levels of 0.09, 0.98 or 10.98 mg ^{14}C-endosulfan /kg body weight. Thereafter the skin was thoroughly washed with a mild soap solution to remove non-absorbed substance. After 24, 48, 72 and 168 hours 4 animals/group were sacrificed and disposition of ^{14}C was measured.
Based on the results of this study the absorption of endosulfan in a typical formulation by the skin of rats goes fairly rapid and is concentration dependent. The dermal penetration of endosulfan amounts to 20% at a high dose of 10 mg/kg after a 10hr dermal exposure. At lower doses the penetration is lower in absolute terms, but higher in terms of percentage, increasing to just over 40%.
However true penetration, i.e. release from the skin into the blood, takes a long time and is the rate-limiting factor. After 24 hours 80% or more of absorbed material was still bound to the skin of rats, while most of the penetrated material (1 - 10%) had been excreted. The maximum penetration occurs after 48 hr in good correlation with peak concentrations found in liver and kidneys at that time point. After one week 95% of the absorbed material had penetrated. More details of this study are presented below.

In vitro penetration of endosulfan through rat and human skin

To reduce the uncertainty of the interspecies extrapolation an “in vitro” study comparing the penetration of endosulfan through excised rat and human skin has been performed (MRID 44863701). In this study the rate of penetration of an experimental ^{14}C-endosulfan-EC33 formulation through isolated human and rat skin was assessed *in vitro* following a single application of 1.0, 0.1 or 0.01 mg endosulfan/cm² to the epidermal surface. Penetration was measured by assessment of radioactivity in duplicate aliquots of receptor-fluid after 1, 2, 4, 8, 10, 16, 24, 48, and 72 hours.

Based on the results of this study the dermal penetration rate through rat skin was a 3.1 – 5.7 times higher than that observed in human skin. More details of this study are presented in below.

Craine, Elliott M. (1988); A Dermal Absorption Study in Rats with ^{14}C-Endosulfan with Extended Test Duration; Wil Research Laboratories Inc. Ohio, USA; Company file No: A39677; Date: 17 November 1988. EPA MRID 41048504

GLP: The study was conducted according to GLP.
Guidelines: The study complies with Test Guideline EPA-FIFRA § 85-3.
Material and Methods : Groups of 16 female Sprague Dawley rats were exposed dermally for 10 hours to a similar-to-field-use EC33 formulation of ^{14}C-endosulfan on a circular area of shorn dorsal skin at actual levels of 0.09, 0.98 or 10.98 mg ^{14}C-endosulfan /kg bw. Thereafter the skin was thoroughly washed with a mild soap solution to remove non-absorbed substance. After 24, 48, 72 and 168 hours 4 animals/group were sacrificed and disposition of ^{14}C was measured.
Findings : No signs of systemic intoxication or any signs of skin irritation were observed. Results are summarized in Table A4. Skin absorption was proportional to the dose, but was in all doses less than 50% and at the high dose (10.98 mg/kg) was only 20%. Only a small amount of residue was still present in the skin after one week, indicating that the penetration process was completed in one week. Some residue also remained in other parts of the body. Concentrations in organs were highest in kidneys and liver and had reached a peak after 48 hours, at which time point the penetration rate had also reached its maximum. Peak concentrations in fat were only half of those in the liver and dissipated much more quickly. The rate of elimination of ^{14}C-material was low at 24 hr, then accelerated with a peak at 48 hours and subsequently slowed down again. Two thirds of the eliminated radioactivity was excreted in the feces and one third in the urine.

Table A6: Disposition of endosulfan in the rat after dermal application

Measured Dose (mg/kg)		0.09	0.98	10.98
Not absorbed	(%)	39.9	54.7	71.8
Absorbed	(%)	46.5	48.0	21.3
Present in skin	(%)	1.7	1.5	1.0
Total penetrated	(% after 24hr)	22.1	16.1	3.8
	(% after 48hr)	35.3	36.2	11.1
	(% after 72hr)	39.0	28.7	12.0
	(% after168hr)	44.8	46.4	20.3
Present in animal	(%)	2.5	2.3	1.3
Excreted Feces	(% after 24hr)	5.6	3.2	0.6
	(% after 48hr)	14.8	13.6	3.2
	(% after 72hr)	21.9	15.2	5.6
	(% after168hr)	28.6	31.1	13.2
Excreted Urine	(% after 24hr)	3.5	2.7	0.7
	(% after 48hr)	7.9	7.2	2.4
	(% after 72hr)	10.4	7.2	2.8
	(% after168hr)	13.7	13.1	5.8
Excreted Total	(% after 24hr)	9.0	5.8	1.4
	(% after 48hr)	22.6	20.6	5.6
	(% after 72hr)	32.4	20.9	8.4
	(% after168hr)	42.3	44.1	19.1

Max. penetration rate	(µg/cm^2/hr)	0.018	0.165	0.532
Total Recovery	(%)	86.4	102.7	93.1

Conclusion: Absorption of endosulfan in a typical formulation by the skin of rats goes fairly rapid and is concentration dependent. The dermal penetration of endosulfan amounts to 20% at a high dose of 10 mg/kg after a 10hr dermal exposure. At lower doses the penetration is lower in absolute terms, but higher in terms of percentage, increasing to just over 40%.
However true penetration, i.e. release from the skin into the blood, takes a long time and is the rate-limiting factor. After 24 hours 80% or more of absorbed material was still bound to the skin of rats, while most of the penetrated material (1 - 10%) had been excreted. The maximum penetration occurs after 48 hr in good correlation with peak concentrations found in liver and kidneys at that time point. After one week 95% of the absorbed material had penetrated.

Noctor, J. C.; John, S. A. (1995). (^{14}C)-Endosulfan; Rates of penetration through human and rat skin determined using an in vitro system; Hazleton Europe, Harrogate, United Kingdom; Company file No: A54103; Date: April 1995. EPA MRID 44863701

GLP: The study was conducted according to GLP.
Guidelines: The study complies with the draft OECD Guideline for 'Percutaneous Absorption: *in vitro* Method'.
Material and Methods: The rate of penetration of an experimental ^{14}C-endosulfan-EC33 formulation through isolated human and rat skin was assessed *in vitro* following a single application of 1.0, 0.1 or 0.01 mg endosulfan/cm² to the epidermal surface. These doses are of the same order as those used for the described above *in vivo* dermal penetration studies on rats.
Frozen intact skin was thawed, cut to uniform thickness resulting in sections of 400 µm. The resulting section consisted of intact epidermis and a portion of dermis. After a check for membrane integrity, a nominal application volume of 64 µl test substance in aqueous solution was added to eight skin sections per dose (12 in the high dose), each mounted in a 'Franz' static *in vitro* dermal penetration cell. Penetrated material was collected in receptor fluid under the skin. This fluid consisted of acidified ethanol/water (1:1) and was held at a temperature of 32°C.
Penetration was measured by assessment of radioactivity in duplicate aliquots of receptor-fluid after 1, 2, 4, 8, 10, 16, 24, 48, and 72 hours. After 72 hours the epidermal surface of all skin-preparations was thoroughly washed with mild detergent. The wash was collected and its radioactivity measured to assess the amount of non-absorbed material. The 4 additional skin-preparations in the high dose groups were given an additional wash after ten hours. Radioactivity in skin was also measured to assess material absorbed but not penetrated. Dermal metabolism was assessed by analysis of metabolites in the receptor fluid.

Table A6.1: ^{14}C-Endosulfan-EC33 Formulation; Comparative 72 hr skin penetration model

Dose (mg/cm²)	0.01		0.1		1.0		1.0 (10hr wash)	
Species	Rat	Man	Rat	Man	Rat	Man	Rat	Man
% not absorbed (72 hr)	1.7	26	3.9	44	23	7	51	59
% present in skin (72 hr)	13.3	7	14.3	13	30	49	28	5
% penetrated (72 hr)	96	61	76	29	40	20	9	4
% penetrated (10 hr)	79	18	35	6	13	7	12	2
% total recovery (72 hr)	111	94	94	87	95	76	88	68
Penetration rate (1-8hr µg/cm²/hr)	0.9	0.2	4.4	0.8	15.9	5.2	15.9	5.2
Penetration Ratio Rat/Man	4.0		5.7		3.1		3.1	

Findings: Penetration of endosulfan through the skin started after a lag time of generally less than one hour at a steadily decreasing rate. The penetration rate was highest between 1 - 8 hr. The penetration rate was dose-dependent and found to be on average 4.3 times higher in rat skin than in human skin. For results see Table A6.1 above. Analysis of the receptor fluid in the high dose groups further revealed interesting differences in

degradation products, showing that human skin has more residual detoxifying capacity than rat skin. This analysis has been summarized below in Table A6.2.

Table A.6.2 14C-Endosulfan-EC33 Formulation; Degradation Products in Receptor Fluid after 72hr

Metabolite	Rat*	Human*
α-Endosulfan	3.1	-
β-Endosulfan	81.4	27.3
Endosulfan-sulfate	2.9	8.3
Endosulfan-diol	8.8	34.0
Endosulfan- OH-ether	-	2.7
Unknown	-	17.2

* Figures indicate percentage of the total radioactivity in receptor fluid

Conclusions: The rate of penetration through human skin is significantly lower than through rat skin. The difference is concentration dependent and the mean ratio rat/man is 4.3. The results of this *in vitro* study are consistent with the results of the *in vivo* dermal penetration study on rats. The skin appears to have a depot function, slowing down the rate of penetration significantly. Longer storage time in the skin may be the basis of the higher degree of dermal detoxification.

Appendix 7

Dikshith T.S.S. et al. (1988)

Effect of Repeated Dermal Application of Endosulfan to Rats

Reprinted from Veterinary and Human Toxicology, Vol. 30, No. 3, June 1988, pp. 219-224

(Full Paper)

APPENDIX 8

Needham, D.; Creedy, C.L.; Hemmings, P.A. (1998) Endosulfan-[C^{14}] Code: AE F002671 00 1E Toxicokinetics in the rat following repeated daily oral administration of 1 mg/kg bodyweight for up to 28 days; AgrEvo UK Limited, Chesterford Park, England; Study No. TOX 97099; Company file No: A67138; Date: 9 April 1998

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Methods, Japan MAFF Guidelines and EPA-FIFRA §85-1
Material and Methods: Male and female Wistar Crl (W1) BR rats were dosed daily with 1 mg endosulfan/kg body weight for up to 28 days. Groups of 4 males and 4 females were killed 24 hours after receiving 1, 10, 16, 22 or 28 doses. At necropsy whole tissue or representative samples of epididymal, subcutaneous and renal fat, kidney, liver, testes (including epididymus, seminal vesicles and prostate), uterus, ovaries, blood and plasma were removed and analyzed for radioactive content. An additional group of rats were killed 2 days after receiving the last of 28 doses.

A further group of rats (4 males + 4 females) were placed into metabowls after receiving 28 doses of endosulfan and urine and feces were collected over the next four days. At necropsy whole tissues or representative samples of the adrenals, blood, bone, brain, eyes, lungs, heart, kidney, liver, muscle, ovaries, plasma, renal fat, skin, spleen, testes (including epididymus, seminal vesicles and prostate), thyroid, subcutaneous fat, uterus, epididymal fat and residual carcass were removed and analyzed for radioactive content.

The final group of 4 male and 4 female rats also received 28 doses of endosulfan. Blood samples were then removed 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 30, 48, 72, 96 and 120 hours after termination of dosing and the radioactive content was determined. At necropsy (5 days after final dose) whole tissue or representative samples of epididymal, subcutaneous and renal fat, kidney, liver, testes (including epididymus, seminal vesicles and prostate), uterus, ovaries, blood and plasma were removed and analyzed for radioactive content.

Findings:

Tissue Residues: There was an increase in the concentration of residues in the tissues of male and female rats following repeated daily oral dosing of 1 mg endosulfan/kg bodyweight. In most tissues the residues reached a maximum value by day 23 with the greatest concentration of radioactivity being found in the kidney (42.7 mg/kg in males and 31.6 mg/kg in females). The concentrations of endosulfan residues in the liver peaked at 4.20 and 6.88 mg/kg in males and females, respectively, but in the remainder of the tissues, the concentration of endosulfan residues were much lower and peaked at 0.24 – 1.21 mg/kg in the case of males rats, and 0.29 – 3.04 mg/kg in female rats. Following a 5 day depuration phase, the concentration of residues fell significantly to levels similar to those seen 24 hours after a single oral dose for most tissues.

Table A8: Mean Concentrations of endosulfan residues in tissues following repeated daily oral administration of 1 mg/kg body weight for up to 28 days (Needham et. al., 1998)

Tissue	**Concentration in males (mg equivalents/kg tissue)**							
Day	**2**	**11**	**17**	**23**	**29**	**30**	**32**	**33[(1)]**
Subcutaneous Fat	0.210	0.537	0.604	0.636	0.502	0.443	0.245	0.196
Epididymal fat	0.223	0.634	0.733	0.737	0.546	0.594	0.361	0.213
Plasma	0.114	0.473	0.260	0.404	0.295	0.196	0.130	0.081
Blood	0.157	0.621	0.766	1.211	1.205	1.055	0.942	0.856
Renal fat	0.216	0.516	0.610	0.550	0.461	0.413	0.236	0.129
Liver	0.704	2.234	2.906	4.200	3.368	2.904	1.707	1.683
Kidney	4.118	23.772	29.349	42.668	40.641	38.188	25.422	27.028
Testes	0.047	0.205	0.194	0.244	0.223	0.195	0.157	0.122
	Concentration in females (mg equivalents/kg tissue)							
Day	**2**	**11**	**17**	**23**	**29**	**30**	**32**	**33[(1)]**
Subcutaneous fat	1.114	3.044	2.923	2.962	2.717	1.786	0.904	0.739
Uterus	0.278	0.858	0.296	0.303	1.219	0.305	0.362	0.189
Plasma	0.055	0.234	0.260	0.293	0.268	0.181	0.113	0.071
Blood	0.056	0.301	0.360	0.473	0.505	0.447	0.345	0.263
Renal fat	1.369	3.960	3.230	3.493	3.932	2.815	1.670	1.092
Liver	0.639	3.248	4.898	6.878	5.772	4.623	3.158	3.202
Kidney	1.630	16.818	22.563	31.265	31.616	29.648	19.136	24.720
Ovaries	0.166	0.758	0.746	0.796	0.601	0.651	0.442	0.340

[1]The slight increase in residue levels in kidney and liver of animals sacrificed at day 33 from animals sacrificed at day 32 was most likely due to study design and housing. The animals sacrificed at day 32 had been in metabolism cages (metabowls) for 4 days and, due to the design of the cages, ate less and drank more than the animals housed in standard holding cages. This change lead to an increase in urinary clearance of polar metabolites, resulting in slightly less residues in liver and kidney.

Excretion profile: The overall recovery of radioactivity, based on the total radioactivity administered over 28 days, was 9.253±0.49% for male rats and 9.79±0.35% for female rats. The kidney and liver contained the greatest amount of dosed radioactivity, equivalent to 0.58 – 0.79 and 0.29 – 0.50%, respectively of the total dose.

Following normalization of the results to convert all recoveries to a percentage of the recovered radioactivity, 12.7±1.7% of the recovered radioactivity was found in the urine and 65.5±3.5% in the feces. When compared to the previous results obtained after administration of a single oral dose, the route and rate of excretion was not significantly affected by repeated administration of endosulfan. Although the percentage of the radioactivity excreted in the feces was reduced due to the considerable amount of endosulfan residues still present in the gastrointestinal tract and tissues at necropsy.

Table A8.1: Excretion of radiolabeled dose, as a percentage of recovered radioactivity, from rats following the last of 28 daily oral doses of 1 mg/kg bodyweight.

Sample	Excretion of radioactivity (as % of recovered radioactivity)								
	Males					Females			
	041M	042M	043M	044M		045F	046F	047F	048F
Urine									
Total after 96 hrs	10.83	12.30	10.83	12.44		13.30	11.56	14.98	15.03
Fecal organic extract									
Total after 96 hrs	3.95	3.47	3.16	5.33		3.24	7.48	2.14	3.55
Fecal aqueous residue (non-organic extractable)									
Total after 96 hrs	65.74	60.97	62.22	64.47		62.33	60.16	58.65	57.19
Cage wash									
Total after 96 hrs	2.16	2.01	2.82	1.50		3.39	3.36	4.20	2.90
Carcass	6.55	7.19	7.18	5.70		5.97	7.22	8.73	8.41
Tissues	10.77	14.06	13.81	10.62		11.77	10.23	11.32	12.92
Overall Total	100.00	100.03	100.02	100.06		100.00	100.00	100.01	100.00

The highest concentrations of radioactivity found in the tissues 4 days after the final dose (Day 32) were in the kidneys with mean values of 25.4 and 19.1 mg/kg for males and females, respectively. The majority of the other tissues contained concentrations of residues below 1.0 mg/kg.

Blood Pharmacokinetics:

Examination of the concentration of radioactive residues in the blood of rats after receiving the last of 28 daily doses of 1 mg endosulfan/kg bodyweight showed that the maximum levels were found 6-8 hours after administration of the final dose. There were sex differences seen with the maximum residue concentration found in the blood of male rats being higher than in female rats (1.48 – 2.05 mg/kg for males and 0.65 – 0.75 mg/kg for females). This is supported by a slower terminal elimination half-life in the male than in the female rats (128.2 – 184.8 hr compared with 91.5 – 111.9 hr).

Conclusions:

Following repeated daily dosing of 1 mg endosulfan/kg bodyweight the concentrations of radioactive residues in all tissues increases with increasing dosing and reached a maximum value within 22 doses in the case of most tissues (i.e. the subcutaneous, renal and epididymal fat, uterus, plasma, blood, liver, kidney, ovaries and testes). Apart from the liver and kidney (the organs of metabolism and excretion) the concentration of endosulfan residues peaked at 0.24 – 1.21 mg/kg in the case of male rats, and 0.30 – 3.04 mg/kg for female rats. The reproductive organs did not contain residue levels greater than the general tissues, neither did they display a greater degree of accumulation of endosulfan residues.

Following cessation of dosing the concentration of radioactive residue levels in al of the tissues fell significantly over the next 5 days to levels that for the most tissues were similar to those seen 24 hours after a single oral dose. The maximum concentration of endosulfan residues in the blood was found to be 1.64 and 0.69 mg/kg for male and female rats, respectively, 6-8 hours after receiving the last dose. The terminal half-life in the blood was found to be 97.75 hours for female rats and 146.6 hours for male rats. The profile of excretion of dosed radioactivity did not appear to be significantly affected by repeated daily administration of endosulfan.